WO03013602

Publication Title:

INHIBITORS OF HER3 ACTIVITY

Abstract:

128d Abstract of WO03013602

The present invention relates to a pharmaceutical composition comprising as an active agent an inhibitor of HER3 activity, particularly an anti-HER3- antibody. Further, the use of this composition for the diagnosis, prevention or treatment of hyperproliferative diseases, particularly tumor diseases is disclosed. Data supplied from the esp@cenet database - Worldwide

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 February 2003 (20.02.2003)

PCT

(10) International Publication Number
WO 03/013602 A1

(51) International Patent Classification[7]: A61K 39/395, 47/48, G01N 33/577, 33/574, A61P 35/00, G01N 33/50, C12N 5/20, C07K 16/28

(21) International Application Number: PCT/EP02/08938

(22) International Filing Date: 9 August 2002 (09.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01 119 260.6 9 August 2001 (09.08.2001) EP

(71) Applicant *(for all designated States except US)*: **MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.** [DE/DE]; Hofgartenstrasse 8, 80539 München (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ULLRICH, Axel** [DE/DE]; Brunnstrasse 5, 80331 München (DE). **HTUN-VAN DER HORST, Edward** [DE/DE]; Blumenstrasse 23, 80331 München (DE).

(74) Agents: **WEICKMANN & WEICKMANN** et al.; Postfach 860 820, 81635 München (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/013602 A1

(54) Title: INHIBITORS OF HER3 ACTIVITY

(57) Abstract: The present invention relates to a pharmaceutical composition comprising as an active agent an inhibitor of HER3 activity, particularly an anti-HER3- antibody. Further, the use of this composition for the diagnosis, prevention or treatment of hyperproliferative diseases, particularly tumor diseases is disclosed.

Inhibitors of HER3 activity

Description

The present invention relates to a pharmaceutical composition comprising as an active agent an inhibitor of HER3 activity, particularly an anti-HER3-antibody. Further, the use of this composition for the diagnosis, prevention or treatment of hyperproliferative diseases, particularly tumor diseases is disclosed.

Protein tyrosine kinases are known to be enzymes, which mediate signal transduction processes that regulate cell growth and differentiation. Receptor protein tyrosine kinases act via ligand-stimulated tyrosine phosphorylation of substrates. HER3 (also called ErbB3) is a member of the epidermal growth factor receptor (EGFR) subfamily of receptor protein tyrosine kinases (Plowman et al., Proc. Natl. Acad. Sci. U.S.A. 87 (1990), 4905-4909; Kraus et al., Proc. Natl. Acad. Sci. U.S.A. 86 (1989), 9193-9197 and Kraus et al., Proc. Natl. Acad. Sci. U.S.A. 90 (1993), 2900-2904).

HER3 has been found to be overexpressed in several types of cancer such as breast, gastrointestinal and pancreatic cancers. When HER3 is co-expressed with HER2, another member of the EGFR subfamily of receptor protein tyrosine kinases, an active heterodimeric signalling complex is formed.

A monoclonal antibody against HER3 (Rajkumar et al., Br. J. Cancer 70 (1994), 459-456) had an agonistic effect on the anchorage-independent growth of cell lines expressing HER3. On the other hand, anti-HER3 antibodies described in U.S. patent 5,968,511 (corresponding to WO 97/35885) are reported to reduce Heregulin-induced formation of

HER2/HER3 heterodimers. Such an activity, however, is only demonstrated for an antibody which increases Heregulin binding to HER3. Thus, it is not clear which type of anti-HER3-antibody - if any - has potential of being used for therapeutic applications.

Vadlamudi et al. (Oncogenes 18 (1999), 305-314) describe the regulation of the cyclooxygenase (COX-2) pathway by the HER2 receptor. It was found that a specific inhibitor of COX-2 can suppress the mitogenic and invasive action of colorectal cancer cells. Further, it was found that incubation with a monoclonal anti-HER3 antibody leads to a reduction in the levels HER2/HER3 heterodimers, but results in an only partial reduction of COX-2 expression.

WO 00/31048 discloses a quinazoline derivative which acts as an inhibitor of receptor tyrosine kinases such as EGFR, HER2 and HER4. An inhibition of HER3 is however not disclosed.

WO 00/78347 discloses methods for arresting or inhibiting cell growth, comprising preventing or reducing ErbB-2/ErbB-3 heterodimer formation. For example, the agent may be a combination of an anti-HER2 extracellular domaine antibody and an anti-HER3 antibody, e.g. the HER3 antibody H3.105.5 purchased from Neomarkers. It is however not clear which type of anti-HER3 antibody is required to obtain desirable therapeutic effects.

US-patent 5,804,396 describes a method for identifying an agent for treatment of a proliferative disorder, comprising the steps of assaying a potential agent for activity in inhibition of signal transdcution by a HER2/HER3 or HER2/HER4 or HER3/HER4 heterodimer. Specific HER3 inhibitors are not dislcosed.

We compared the biological properties of Herceptin, an agonistic monoclonal antibody against HER2 with anti-HER3-antibodies, namely (i) α-

HER3-ECD, a murine monoclonal antibody IgG1, Upstate Biotechnology, Cat.No.# 05-471, directed against the Heregulin binding site of HER3, (ii) antibody 1B4C3 from our laboratory and (iii) antibody 2D102 also from our laboratory, in invasive breast cancer cell lines MCR-7 (DKFZ Heidelberg), MDA-MB-468 (ATCC HTB-132) and MDA-MB231 (ATCC HTB-26) expressing different HER2:HER3 ratios. We provide evidence that pretreating the breast cancer cell lines with anti-HER3-antibody prior to α/ß-Heregulin (α/ß-HRG) stimulation diminished the HER2/HER3 tyrosine phosphorylation content in contrast to Herceptin. In addition, anti-HER3-antibody abrogated HER2/HER3 heterodimerization and also reduced the complex formation of the p85 subunit of PI_3-kinase and the adaptor protein SHC with HER3, resulting in decreased PI_3-kinase and c-jun-terminal kinase activity (JNK), respectively. In comparison to Herceptin, anti-HER3-antibody was also capable of downregulating extracellular signal-regulated kinase 2 (ERK2) after α/ß-HRG stimulation. Furthermore, we demonstrate a significant reduction of the migratory and proliferative property of the breast cancer cell lines after pretreatment with anti-HER3-antibody. Our data clearly show that αn anti-HER3-antibody is more potent in diminishing signal transduction processes after HRG stimulation than Herceptin. Furthermore, in specific cancer types, e.g. melanoma, anti-HER3 antibodies are effective in reducing migratory and proliferative properties while anti-HER2 antibodies do not show any significant effect at all. These data demonstrate the great potential of anti-HER3 antibodies or other HER3 inhibitors for the therapy of breast cancer and other malignancies characterized by hypersignalling through HER3 and its heterodimerization partners.

Thus, the present invention relates to a pharmaceutical composition comprising as an active agent a specific type of inhibitor of HER3 activity and pharmaceutically acceptable carriers, diluents and/or adjuvants. The HER3 inhibitor of the invention is characterized in that binding of the inhibitor to HER3 reduces HER3 mediated signal transduction. In one

embodiment a reduction of HER3 mediated signal transduction may be caused by a downregulation of HER3 resulting in an at least partial disappearance of HER3 molecules from the cell surface. In a further embodiment of the invention the reduction of HER3 mediated signal transduction may be caused by a stabilization of HER3 on the cell surface in a substantially inactive form, i.e. a form which exhibits a lower signal transduction compared to the non-stabilized form.

The inhibitor of the invention may influence the binding of Heregulin to HER3, particularly by decreasing the binding of Heregulin to HER3. In other embodiments, however, the inhibitor may not compete with the binding of Heregulin to HER3.

In a preferred embodiment the inhibitor is an anti-HER3-antibody. Preferably, the antibody is directed against the extracellular domain of HER3. It should be noted, however, that also other HER3 inhibitors, particularly low molecular weight inhibitors, e.g. peptides or organic compounds may be used.

According to the invention, the term "antibody" covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two antibodies and antibody fragments as long as they exhibit the desired activity.

The antibody may be a monoclonal antibody which may be obtained by the hybridoma method as described by Köhler et al. (Nature 256 (1975), 495) or by recombinant DNA methods (cf. e.g. U.S.Patent 4,816,567). Monoclonal antibodies may also be isolated from phage antibody libraries using techniques described in Clackson et al. (Nature 352 (1991), 624-628) and Marks et al. (J.Mol.Biol.222 (1991), 581-597). The antibody may be an IgM, IgG, e.g. IgG1, IgG2, IgG3 or IgG4.

Antibody fragments comprise a portion of an antibody, generally the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments, diabodies, single chain antibody molecules and multispecific antibody fragments.

Particularly, the antibody may be a recombinant antibody or antibody fragment, more particularly selected from chimeric antibodies or fragments thereof (Morrison et al., Proc. Natl. Acad. Sci. U.S.A. 81 (1984), 6851-6855), humanized antibodies (Jones et al., Nature 321 (1986), 522-525; Riechmann et al., Nature 332 (1988), 323-329 and Presta, Curr. Op. Struct. Biol. 2 (1992), 593-596), single chain Fv antibodies (Plücktuhn in: The Pharmacology of Monoclonal Antibodies 113, Rosenburg and Moore, EDS, Springer Verlag, N.Y. (1994), pp. 269-315) and diabodies (Hollinger et al., Proc. Natl. Acad. Sci. U.S.A. 90 (1993), 6444-6448).

In an especially preferred embodiment the antibody is selected from antibodies 1B4C3 (IgG2a) and 2D1D12 (IgG1) produced by the hybridoma cell lines DSM ACC 2527 or DSM ACC 2517, fragments thereof or recombinant derivatives thereof. 1B4C3 is an antibody which leads to internalization of HER3 and 2D1D12 is an antibody which leads to stabilization of HER3. Further preferred are antibodies, e.g. chimeric or humanized antibodies or fragments thereof, which have substantially the same biological activity (e.g. as described in the Examples) compared to the antibodies produced by the deposited hybridoma cell lines, for example, by binding to the same epitope on HER3. The hybridoma cell line DSM ACC 2517 was deposited under the Budapest Treaty for the Deposit of Microorganisms on July 24, 2001 at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Mascheroder Weg 1b, 38124 Braunschweig, Germany. The hybridoma cell line DSM ACC 2527 producing the antibody 1B4C3 was deposited on August 07, 2001 at DSMZ.

The antibody of the invention may be coupled to a labelling group, particularly for diagnostic applications. Examples for suitable labelling groups such as radioactive groups, fluorescent groups or other labelling groups are known in the art. Further, particularly for therapeutic applications, the antibody may be coupled to an effector group, e.g. a cytotoxic group such as a radioactive group, a toxin or another effector group as known in the art.

In a further preferred embodiment, the inhibitor may be selected from non-antibody derived binding proteins such as fibronectin type III domains or anticalins (Skerra, "Engineered protein scaffolds for molecular recognition", J. Mol. Recog. 13 (2000), 167-187 and references cited therein).

Further, the present application relates to the use of an inhibitor of HER3 activity, wherein binding of said inhibitor to HER3 reduces HER3 mediated signal transduction, for the manufacture of an agent for the diagnosis, prevention and/or treament of hyperproliferative diseases, particularly tumor diseases such as breast cancer, gastrointestinal cancer, pancreas cancer, prostate cancer, glioma, melanoma or other HER3 expressing or overexpressing cancers or formation of tumor metastases. The disease may be associated with increased HER3 signal transduction and may be associated with concomittant HER2 expression or lack of HER2 expression. Particularly the disease is associated with increased HER3 phosphorylation and/or increased HER2/HER3 heterodimerization and/or increased PI_3 kinase activity and/or increased c-jun terminal kinase activity and/or AKT activity and/or increased ERK2 activity and/or PYK2 activity.

Surprisingly it was found that the HER3 inhibitor of the invention, particularly an anti-HER3-antibody, shows a significantly higher efficiency in diminishing signal transduction processes than a HER2 inhibitor such as Herceptin. Particularly, in melanoma cells, the anti-HER3-antibody was

effective, while Herceptin did not show a significant effect, even though HER2 was expressed by the melanoma cells.

Preferably, the HER3 inhibitor of the invention exhibits at least one of the following characteristics:

- decreasing the association of Heregulin (p85) with transactivated HER3, preferably substantially completely inhibiting the binding of p85 with HER3,
- inhibiting the binding of GRB2 to HER2, the binding of HER2 to HER3 and/or the association of GRB2 with SHC,
- inhibiting receptor tyrosin phosphorylation,
- inhibiting AKT phosphorylation,
- decreasing tumor invasiveness, particularly in breast cancer and melanoma,
- inhibiting PYK2 tyrosine phosphorylation and
- inhibiting ERK2 phosphorylation.

Further, the invention relates to a method for diangosing, preventing or treating a hyperproliferative disease, particulary a tumor disease, comprising administering a subject in need thereof, e.g. a human, an effective amount of an inhibitor of HER3 activity, wherein binding of said inhibitor to HER3 reduces HER3 mediated signal transduction.

The HER3 inhibitor, particularly the anti-HER3-antibody may be formulated by mixing the active agent with physiologically acceptable carriers, diluents and/or adjuvants, e.g. in the form of lyophilized formulations, aqueous solutions, dispersions or solid preparations such as tablets, dragees or capsules as described in Remington's Pharmaceutical Sciences.

The formulation may also contain more than one active compound, e.g. inhibitors of other receptor protein tyrosine kinases such as EGFR, HER2,

HER4 or vascular endothelial factor (VEGF). Alternatively or additionally, the composition may comprise a cytotoxic agent such as doxorubicin, cis-platin or carboplatin, or a cytokine.

The inhibitor of the invention is also suitable for diagostic applications, e.g. in order to determine the expression and/or activity of HER3 on target cells. Such a diagnostic application may be carried out according to known procedures.

Depending on the type and severity of the disease to be treated, about 1 μg/kg to 15 mg/kg of antibody may be administered to a human patient, e.g. by one or more separate administrations or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to about 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition to be treated, the treatment is sustained until a desired suppression of disease symptoms occurs.

Further, the present invention shall be explained by the following figures and examples:

Examples

1. Monoclonal antibody α-HER3^{ECD} decreases receptor tyrosine phosphorylation of HER3 and HER2

The breast cancer cell lines MCF-7 (DKFZ - Heidelberg), MDA-MB-468 (ATCC HTB-132) and MDA-MB-231 (ATCC HTB-26) were chosen on the basis of their different ratios of HER2:HER3 and their inherent migratory properties with MDA-MB-231 being the most invasive cell line. In order to assess the functional role of α-HER3^{ECD} (Upstate Biotechnology, Cat. # 05-471) in comparison to trastuzumab (Herceptin), we pretreated the cells

with α-HER^{ECD} and HC, respectively, prior to Heregulin (HRG) stimulation, performed receptor-immunoprecipitation experiments and probed with an anti-phosphotyrosine antibody (PY) (Figure 1). Our data show that pretreatment with α-HER3^{ECD} substantially decreased the tyrosine phosphorylation content of HER3 and HER2 after α-HRG stimulation in MCF-7 (Figure 1a) and MDA-MB-231 (Figure 1c), but conversely increased HER3 tyrosine phosphorylation in MDA-MB-468 (Figure 1b). The association between HER2 and HER3 was even enhanced with α-HER3^{ECD}, although the content of tyrosyl-phosphorylated receptors was dramatically reduced (Figure 1 a, b middle upper panel lanes 4 and 8). In contrast, HC upregulated receptor tyrosyl-phosphorylation and promoted association of HER3 and HER2 in the presence or absence of HRG in all cell lines (Figure 1a, b, c upper panel lanes 3, 7, 11 and 15). In the case of MDA-MB-486 cells, which are insensitive to α-HRG, ß-HRG was used as stimulus.

2. α-HER3^{ECD} abrogates association of SHC and PI_3-K with HER3 and of GRB2 with HER2

We subsequently asked whether α-HER3^{ECD} has an effect on the known substrates of HER3, namely SHC and phosphatidyl-3-OH-kinase (PI_3-K), which are effector proteins responsible for MAPK cascade activation and lipid signalling, respectively. Therefore, we immunoprecipitated SHC and PI_3-K under the experimental conditions described above and assessed the tyrosine phosphorylation of these effectors. As shown in Figure 2, α-HER3^{ECD} significantly descreased the tyrosine phosphorylation of SHC in the cell lines MCF-7 and MDA-MB-486 (Figure 2a, b compare lane 13 with 16). Interestingly, the association of SHC was attenuated in MCF-7 cells, whereas in MDA-MB-486, α-HER3^{ECD} lead to increased binding of HER3 with SHC. The immunoprecipitates of the regulatory subunit of PI_3-K yielded essentially similar results. The binding of tyrosine-phosphorylated HER3 to PI_3-K was abrogated in MCF-7, while an increase was observed in

MDA-MB-486 (Figure 1b, 2b). However, pretreatment with α-HER3^{ECD} in MDA-MB-486 cells lead to increased binding of SHC and PI_3-K to HER3, whereas HC again showed crosslinking properties in all cell lines. Since SHC associates with the adaptor molecule GRB2 after HRG stimulation, we explored the effect of the reduced tyrosyl-phosphorylation of SHC by measuring GRB2 binding (Figure 2c). Therefore we performed GST-pulldown assays in MCF-7 cells using GST-GRB2 fusions and the same experimental design as before. Indeed, the reduced tyrosyl-phosphorylation of SHC resulted in strongly decreased binding of GRB2 to SHC (Figure 2c lower panel, compare lanes 5 and 8), and a complete inhibition of its association with HER2 (Figure 2c middle panel, compare lanes 5 and 8). These data clearly show that α-HER3^{ECD} inhibits SHC and PI_3-K binding to HER3 in MCF-7, but conversely in MDA-MB-486 both proteins associated with HER3 regardless of the phosphorylation status of HER3.

3. α-HER3^{ECD} downregulates JNK1 and PI_3-K activity

The adaptor protein SHC mediates MAPK signalling pathways downstream of growth-factor receptors, activating ERK2 and JNK, resepectively. To investigate the effect of α-HER3^{ECD} on ERK2 and JNK, we performed MAPK kinase assays under the same experimental conditions in MCF-7 and MB-468 (Figure 3). We observed a strong decrease of JNK activity in all cell lines, but an equivalent effect of HC on JNK was only detectable in MCF-7 (Figure 3a). ERK2 activity was only slightly but significantly decreased through α-HER3^{ECD}, whereas HC had no effect on ERK2 activity (data not shown). Since an involvement of PI_3-K in carcinoma invasion has recently been demonstrated, we investigated the inhibitory properties of α-HER3^{ECD} on PI_3-K activity and carried out PI_3-K assays (Figure 3). In MCF-7 and MDA-MB-486 PI_3-K activity was strongly reduced in comparison to HRG-treated cells (Figure 3a, b). In MDA-MB-486 HC possessed an even greater effect on PI_3-K activity than α-HER3^{ECD}. These data suggest that α-HER3^{ECD}

downregulates JNK and PI_3-K activity, respectively, in MCF-7 and MDA-MB-486 cells.

4. α-HER3^{ECD} enhances endocytotic downregulation of HER3

HER2 and HER3 are endocytosed and recycled after HRG stimulation. We were interested in establishing whether α-HER3^{ECD}-mediated inhibition of HER3 tyrosyl-phosphorylation originates from accelerated endocytosis. To gain insight, we performed a time course with MCF-7 cells in the absence or presence of α-HER3^{ECD} or HRG, respectively, and stimulated subsequently with HRG (Figure 4). HER3 was then immunoprecipitated after biotinylation of the membrane proteins. We observed that HER3 is endocytosed rapidly after pretreatment with α-HER3^{ECD} (Figure 4b upper panel). Applying HRG to the cells had the same effect, with the difference that after two hours HER3 was exported back to the membrane and after three hours it was endocytosed again. As a control, whole cell lysates (WCL) were probed with PY (Figure 4b lower panel). In comparison to HRG-treated cells where the content of tyrosyl-phoshorylated protein was diminished after three hours, accelerated endocytosis of HER3 occured after one hour of pretreatement with α-HER3^{ECD}. To compare α-HER3^{ECD} with HC we performed the same experiment with HC and immunoprecipitated HER2 (Figure 4a upper panel). Strikingly, HER2 was not endocytosed after pretreatement with HC at any time point, whereas HRG lead to rapid endocytosis. When endocytosed receptors and whole cell lysates, probed with anti-phosphotyrosine (PY) are compared, it clearly appears that the phosphotyrosine content decreased with α-HER3^{ECD}, but not with HC (Figure 4b lower panel). Our data indicate that α-HER3^{ECD} downregulates HER3 rapidly through accelerated endocytosis, thus rendering the cell insensitive to HRG stimulation.

5. α-HER3^{ECD} inhibits migratory and proliferative properties of breast cancer cell lines

In order to assess the biological function of α-HER3^{ECD} on the migratory and proliferative properties of breast cancer cells, we performed BrdU-incorporation assays in the presence or absence of α-HER3^{ECD} and stimulated with HRG. Pretreatment with α-HER3^{ECD} decreased proliferation by 28.7% ± 6.18% and 21.1% ± 7.62% in MCF-7 and MDA-MB-486, respectively. The observed inhibition in proliferation correlated with the ERK2 assays, whereas HC had no effect in these cell lines (data not shown). Furthermore, to investigate the effect of α-HER3^{ECD} on the migratory properties of breast cancer cells, we conducted chemotaxis experiments with MCF-7 and MDA-MB-486 in the presence or absence of α-HER3^{ECD}. We observed a strong decrease in migration of 59.1% ($P=0.018$) and 55.4% ($P=0.00005$) in MCF-7 and MDA-MB-486, respectively. In addition, migration could also be inhibited in MDA-MB231 by 35%, but with a lesser significance ($P=0.06$). Our data clearly show an inhibitory effect of α-HER3^{ECD} on proliferation and migration in MCF-7 and MDA-468.

6. Generation of monoclonal antibodies against HER3

We generated then murine monoclonal antibodies against the extracellular domain of HER3, immunizing Balb/c mice with a human recombinant fusion protein of the extracellular part of HER3 and a C-terminal His-Tag (HER3-6xHis-CT). The immunogen was obtained by transfection, selection with G418 and stable expression of the construct in HEK293 cells; the cell culture supernatant of the clone with the highest expression level was collected and the protein purified after ammonium sulfate precipitation, dialysis and subsequent metal ion affinity chromatography (Ni-NTA). Quality assurance was accomplished by Western blotting (data not

shown). Immunization was performed by intraperitoneal injection with 22 μg of HER3-6xHis-CT according to the manufacturer's protocol (Qiagen ImmunEasy Mouse Adjuvant). Hybridoma cell lines producing monoclonal antibodies were generated using standard methods.

7. Monoclonal antibodies against HER3 preferentially bind to its protein backbone and have different effects on the endocytic processes of HER3

We identified by FACS analysis three monoclonal antibodies recognizing specifically native HER3 on the cell surface of MCF-7 cells (data not shown). 1B4C2 and 1B4C3 are IgG2a isotype antibodies, whereas 2D1D12 is an IgG1 isotype antibody. No cross-reactivity with the other members of the EGFR family was detected (data not shown). We then wanted to determine whether the antibodies bind to glycosylated structures or to the protein backbone of HER3 and which consequences this would have on the endocytic regulation of the receptor. Therefore we pretreated MCF-7 cells in the presence or absence with the antibiotic Tunicamycin for 16 h, which is known to prevent N-linked glycosylation of cell surface proteins. After lysing the cells we immunoprecipitated HER3 with the monoclonal antibodies 2F12 (directed against the intracellular part of HER3), α-HER3^{ECD} (extracellular part of HER3), 1B4C2, 1B4C3 and 2D1D12. Our data show that α-HER3^{ECD}, 1B4C3 and 2D1D12 all bind preferentially to the protein backbone of HER3, whereas 1B4C3 also has an affinity to glycosylated forms of HER3 (Figure 6A).

To investigate the effect of 1B4C3 and 2D1D12 on the endocytic processes of HER3, we performed a time-course experiment, wherein MCF-7 cells where incubated for various time periods with 1B4C3 or 2D1D12, respectively. The cell surface proteins were biotinylated, precipitated with an antibody against the intracellular domain of HER3 and probed against streptavidin. We observed that 1B4C3 accelerates the endocytosis of HER3

similarly to α-HER3^{ECD}, whereas 2D1D12 stabilized and therefore accumulated HER3 on the cell surface (Figure 6B).

8. Monoclonal antibodies 1B4C3 and 2D1D12 inhibit downstream signals of HER3 and HER2

We then asked whether 1B4C3 and 2D1D12 could inhibit tyrosine phosphorylation of HER2 and of the HER3 substrate SHC. Since GRB2 binds to HRG-stimulated HER2 and transmits in the same way as SHC mitogenic signals to the MAPK pathway, we immunoprecipitated SHC and in parallel performed a GST-GRB2 pulldown in MCF-7 cells untreated or pretreated with the antibodies and subsequently stimulated with HRG (Figure 6C). This experiment shows that both antibodies inhibit tyrosine phosphorylation of SHC and the association of GRB2 with SHC. Furthermore, the antibodies inhibit the association of SHC with HER3 and the heterodimerization between HER2 and HER3. These data show that downstream signalling is inhibited by 1B4C3 and 2D1D12, albeit with 2D1D12 having an even stronger inhibitory effect than 1B4C3.

9. Monoclonal antibody 2D1D12 inhibits proliferation of breast cancer cell lines MDA-MB-435S, ZR-75-1 and melanoma cell line Mel Gerlach

We next set out to explore the biological activity of 1B4C3 and 2D1D12 in the two breast cancer cell lines MDA-MB-435S (ATCC HTB-129), ZR-75-1 (ATCC CRL-1500) and the melanoma cell line Mel Gerlach (Klinikum Großhadern, Munich). We have chosen the cell lines due to their tumorigenicity in nude mice and their high HER3 expression level. It should be noted that melanoma cells overexpress HER3, since HER3 is critical in the development of melanocytes as well as oligodendrocytes. To test our hypothesis that 1B4C3 and 2D1D12 abrogate mitogenic signals and consequently the proliferative properties of cancer cells, we performed

BrdU-incorporation assays in the presence or absence of the antibodies (Figure 7). Proliferation was strongly reduced in all cell lines by 2D1D12, whereas 1B4C3 had only an inhibitory effect in Mel Gerlach. Taken together, our data constitute the first evidence that monoclonal antibodies against HER3 could be potentially regarded as new therapeutic weapons against cancer.

The hybridoma cell lines producing antibodies 1B4C3 and 2D1D12 were deposited on August 07, 2001 and July 24, 2001, respectively, at DSMZ.

10. Effect of HER3 antibodies on signal transduction

10.1. Methods

MDA-MB-435S were obtained from ATCC (HTB-129) and Mel-Juso were obtained from Cell Lines Servive (CLS) (0282-HU). GST-p85 (a.a 333-430) was obtained from Santa Cruz. GST-GRB2 was purified as described previously. Phospho-AKT (P-Ser 473) was from New England Biolabs (NEB). HER2 antibody was purified as described from the hybridoma culture supernatent (ATCC CRL-10463). For GST-pull-down assays 1.25 µg bait protein was used. BrdU-Incorporation and invasion assays were performed as previously described. All experiments were performed at least two times.

10.2. Results and Discussion

In order to examine the surface expression of HER2 and HER3 receptors in MDA-MB-435S and Mel-Juso, we additionally determined their expression level by FACS analysis (Fig. 8A, B). We observed HER2 and HER3 expression in these cell lines and went on to dissect the molecular mechanism by which HER3 antibodies act on Heregulin (HRG) mediated signal transduction. Therefore, we performed GST-pull-down assays in the

human breast carcinoma cell line MDA-MB-435S and melanoma cell line Mel-Juso (Fig. 8). Quiescent cells were pretreated with HER3 antibodies 1B4C3, 2D1D12, the control anti-HER2 antibody and with PI(3)K inhibitor LY294002 and were subsequently stimulated with β-HRG. After cell lysis, protein levels were normalized and since HER3 has six potential p85 binding sites a GST-pull-down assay with GST-p85 (a.a. 333-430) as bait was performed. Western blot against phosphotyrosine (PY), reveals that anti-HER2 and 1B4C3 equally decrease p85 association with transactivated HER3, while LY294002 (negative control) has no inhibitory effect on p85 binding in MDA-MB-435S (Fig. 8C). However, 2D1D12 almost completely abrogates binding of p85 with HER3 in MDA-MB-435S (Fig. 8C).

In the human melanoma cell line Mel-Juso 1B4C3 and 2D1D12 equally decrease p85 association with HER3, while anti-HER2 exhibits a more pronounced decrease in receptor association of p85 (Fig. 8D). Again, LY294002 showed no inhibitory effect on p85 binding (Fig. 8D). Moreover, we observed some prominent tyrosine phosphorylated bands in the phosphotyrosine blot at 125kDa and 66kDA in MDA-MD-435S and only a major band at 125kDA in Mel-Juso. It is known that PI(3)K associates physically with focal adhesion kinase (FAK) and therefore we reprobed the blot with FAK antibodies (Fig. 8C, D lower panels). Our data show that in both cell lines only 2D1D12 and the PI(3)K inhibitor LY294002 abolished FAK association with p85. Reprobing the blot with HER2 and HER3 antibodies confirmed the diminished amount of captured HER3 and its reduced heterodimerization with HER2 (Fig. 8C, D middle panels). Taken together, our data indicate that although HER3 and HER2 antibodies decrease receptor tyrosine phosphorylation levels, they are capable of modulating different responses at the secondary level of receptor-associated effector proteins.

Since GRB2 only binds directly to HER2 and indirectly over SHC for HER3, we performed additional GST-pull-down experiment with GST-GRB2 as bait

under identical experimental conditions and human tumor cell lines as above (Fig. 9A, B). We observed that 1B4C3 and 2D1D12 diminished receptor tyrosine phosphorylation between 160 and 185kDa, whereas LY294002 had no ihibitory effect (Fig. 9A, B upper panel). However, pretreatment of the cells with anti-HER2 antibodies lead to increased tyrosine phosphorylation of the receptors, which could be further potentiated with β-HRG. Reprobing with HER2, HER3 and SHC antibodies show that 1B4C3 and 2D1D12 substantially inhibit GRB2 binding with HER2 and its indirect association with HER3 (Fig. 9A, B middle panels), as well as its association with SHC in both cell lines (Fig. 9A, B lower panels). On the other hand, anti-HER2 antibodies increased GRB2 binding to HER2 and SHC.

To gain more insight into antibody-mediated downstream signaling, we also analysed whole cell lysates (WCL) of the experiments described above (Fig. 10). When we looked at the phosphoprotein content of total cellular protein we observed that in both cell lines anti-HER2 constitutively activated the receptors, whereas 1B4C3 and 2D1D12 inhibited tyrosine phosphorylation of the receptors (Fig. 10A upper panel). Again LY294002 had no effect.

It is well established that HRG activates the mitogen-activated protein kinases (MAPK) pathway, leading to cell proliferation, cell survival and enhanced transcription of various genes. To examine the effect of HER2 and HER3 antibodies on HRG-induced MAPK activation, immunoblots of MDA-MB435S and Mel-Juso whole cell extracts were probed with phospho-ERK (T202/Y204) antibodies (Fig. 10). Phosphorylation of the MAPK ERK1/2 (p44/p42) showed, that despite activating the receptor, anti-HER2 slightly decreased ERK1 phosphorylation, whereas 1B4C3 and 2D1D12 had no inhibitory effect on ERK1 phosphorylation (Fig. 10A middle panel). Further reprobing the blot confirmed equal amounts of loaded protein (Fig. 10A lower panel).

Furthermore, we investigated the activation status of AKT, which is a downstream target of PI(3)K and has an important role in cell survival. We observed that anti-HER2, 1B4C3 and 2D1D12 markedly inhibited AKT phosphorylation in Mel-Juso melanoma cells (Fig. 10B upper panel). In MDA-MB-435S breast cancer cells both HER2 and HER3 antibodies significantly decreased AKT phosphorylation (Fig. 10C). LY294002 served as the positive control. This observation is of major importance, since breast cancer patients with markedly increased expression of activated AKT are more prone to relapse with distant metastasis resulting in poor clinical outcome (Perez-Tenorio G et al. British Journal of Cancer, 86, 540-545 (2002).

Monoclonal antibodies 1B4C3 and 2D1D12 inhibit proliferation and migration of breast cancer cell lines MDA-MB-435S and melanoma cell line Mel-Juso

To evaluate the inhibitory function of 1B4C3 and 2D1D12 on cell cycle progression and tumor invasion, we performed BrdU-Incorporation and invasion assays (Fig. 11).

We saw a robust decrease in β-HRG stimulated BrdU-incorporation in MDA-MB-435S and Mel-Juso cells pretreated with 2D1D12 (Fig. 11A). Invasion assays revealed that anti-HER3 antibodies 2D1D12 and 1B4C3 substantially decreased invasiveness of MDA-MB-435S breast cancer and Mel-Juso melanoma cells. Surprisingly HER2 antibody 4D5 only showed an inhibition in MDA-MB-435S but not in the melanoma cell line Mel-Juso although the receptor is expressed at the cell surface (Fig. 11B, C and Fig. 8A, B). Our results suggest the use of anti-HER3 antibodies for the treatment of breast cancer and melanoma.

Monoclonal antibody 2D1d12 inhibits Heregulin-stimulated phosphorylation of PYK2

We previously demonstrated that the intracellular tyrosine kinase PYK2 associates with and is phosphorylated by HER3, suggesting that PYK2 functions as a mediator of HER3 activities. Consistent with this, dominant-negative PYK2 inhibited HRG-mediated invasion of glioma cells. Therefore, we wanted to explore the effect of anti-HER3 antibodies on HRG-induced PYK2 tyrosine phosphorylation. We pretreated quiescent SF767 human glioma cells with anti-HER2, 1B4C3 and 2D1D12 and subsequently stimulated the cells with α-HRG. After lysis and normalising for equal protein amounts, we immunoprecipitated PYK2 and blotted against phosphotyrosine (PY). We observed that whereas anti-HER2 and 1B4C3 had no inhibitory effect on tyrosine phosphorylation of PYK2, 2D1D12 markedly decreased PYK2 tyrosine phosphorylation (Fig. 12A). Thus, anti-HER3 antibodies are effective in inhibiting HRG-induced tyrosine phosphorylation of PYK2.

By probing immunoblots of WCL with phospho-ERK antibodies we observed that pretreating the cells with anti-HER2, 1B4C3 and 2D1D12 inhibited α-HRG activated ERK2 phosphorylation (Fig. 12B middle panel). Reprobing with ERK antibodies confirmed equal amount of loaded protein (Fig. 12B lower panel). Again, our data show that HER3 antibodies downregulate HRG-mediated signaling events in MDA-MB-435S, Mel-Juso and SF767. Furthermore, our analysis suggests that antibodies directed against ectodomains of HER2 and HER3 modulate differential signaling, leading to distinct responses of downstream effector proteins.

Claims

1. A pharmaceutical composition comprising as an active agent an inhibitor of HER3 activity and pharmaceutically acceptable carriers, diluents and/or adjuvants, wherein binding of said inhibitor to HER3 reduces HER3 mediated signal transduction.

2. The composition of claim 1, wherein said reduction of signal transduction is caused by a down-regulation of HER3.

3. The composition of claim 1, wherein said reduction of signal transduction is caused by a stabilization of HER3 in a substantially inactive form.

4. The composition of any one of claims 1-3, wherein the inhibitor does not compete with the binding of Heregulin to HER3.

5. The composition of any one of claims 1-4, wherein said inhibitor is an anti-HER3-antibody.

6. The composition of claim 5, wherein said antibody is a monoclonal antibody or a fragment thereof.

7. The composition of claim 5, wherein said antibody is a recombinant antibody or antibody fragment.

8. The composition of claim 7, wherein said recombinant antibody or antibody fragment is selected from chimerized antibodies, humanized antibodies, single chain antibodies and fragments thereof.

9. The composition of claims 5-8, wherein said antibody is coupled to a labelling group or an effector group.

10. The composition of claims 5-9, wherein the antibody is selected from antibodies 1B4C3 and 2D1D12 produced by hybridoma cell lines DSM ACC 2527 or DSM ACC 2517, fragments thereof or recombinant derivatives thereof.

11. The composition of any one of claims 1-10 comprising a further active agent.

12. The composition of any one of claims 1-11 for diagnostic applications.

13. The composition of any one of claims 1-11 for therapeutic applications.

14. Use of an inhibitor of HER3 activity defined as in any one of claims 1-10 for the manufacture of an agent for the diagnosis, prevention or treatment of hyperproliferative diseases, particularly tumour diseases.

15. The use of claim 14 wherein said inhibitor is an anti-HER3 antibody.

16. The use of claim 15, wherein the antibody is selected from antibodies 1B4C3 and 2D1D12 produced by hybridoma cell lines DSM ACC 2527 or DSM ACC 2517, fragments thereof or recombinant derivatives thereof.

17. The use of any one of claims 14-16 for the diagnosis, prevention or treatment of breast cancer, gastrointestinal cancer, pancreas cancer, prostate cancer, glioma, melanoma or formation of tumor metastases.

18. The use of any one of claims 14-17, wherein said disease is associated with increased HER3 phosphorylation.

19. The use of any one of claims 14-18, wherein said disease is associated with increased HER2/HER3 heterodimerization.

20. The use of any one of claims 14-19, wherein said disease is associated with an increased activity of PI_3-kinase, c-jun-terminal kinase, AKT, ERK2 and/or PYK2.

21. A method for diagnosing, preventing or treating a hyperproliferative disease, particularly a tumour disease comprising administering a subject in need thereof an effective amount of an inhibitor of HER3 activity defined as in any one of claims 1-10.

22. The method of claim 21, wherein said inhibitor is an anti-HER3-antibody.

23. The method of claim 22, wherein the antibody is selected from antibodies 1B4C3 and 2D1D12 produced by hybridoma cell lines DSM ACC 2527 or DSM ACC 2517, fragments thereof or recombinant derivatives thereof.

24. The method of any one of claims 21-23, wherein said subject is a human.

25. A method for identifying a novel agent for diagnosing, preventing or treating a hyperproliferative disease, particularly a tumour disease comprising assaying if a candidate compound is capable of inhibiting HER3 activity, wherein binding of said inhibitor to HER3 reduces HER3 mediated signal transduction.

26. The method of claim 25, wherein an HER3 inhibitor or a compound derived therefrom is formulated as a pharmaceutical composition.

27. Hybridoma cell line DSM ACC 2517 and cell line derived therefrom.

28. Hybridoma cell line DSM ACC 2527 and cell line derived therefrom.

29. Antibody produced by a cell line of claim 27 or 28.

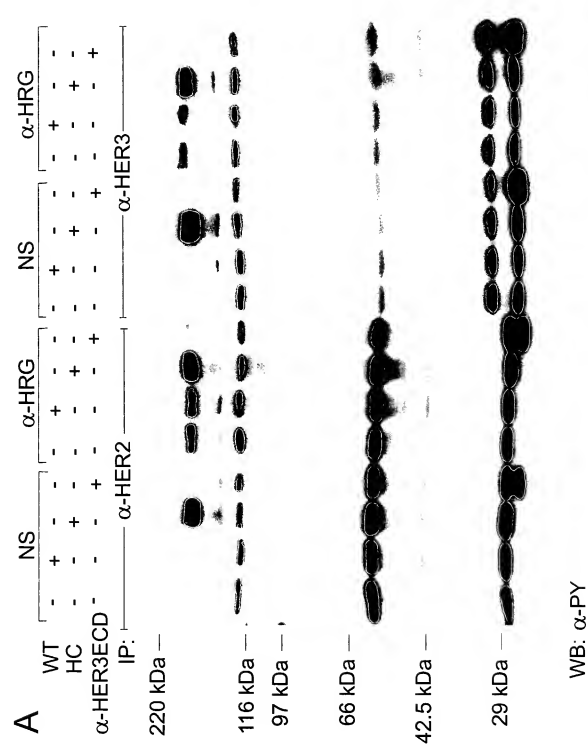


Figure 1
Htun van der Horst et al.

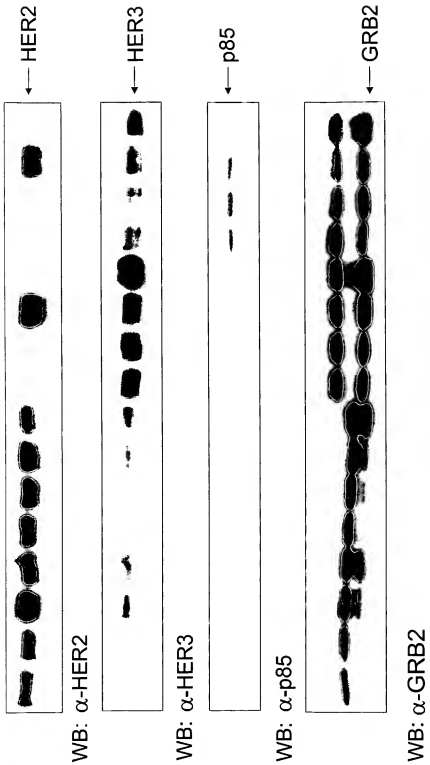


Figure 1
Htun-van der Horst et al.

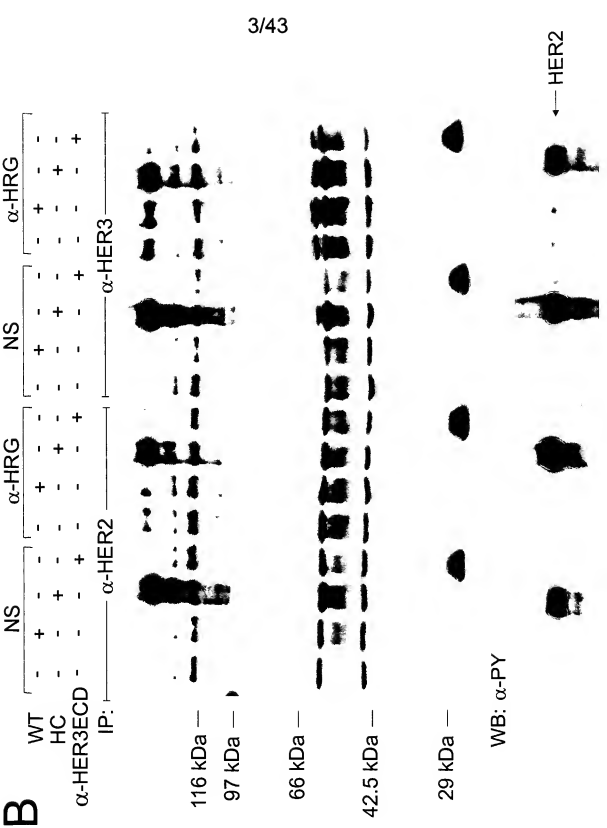


Figure1
Htun-van der Horst et al.

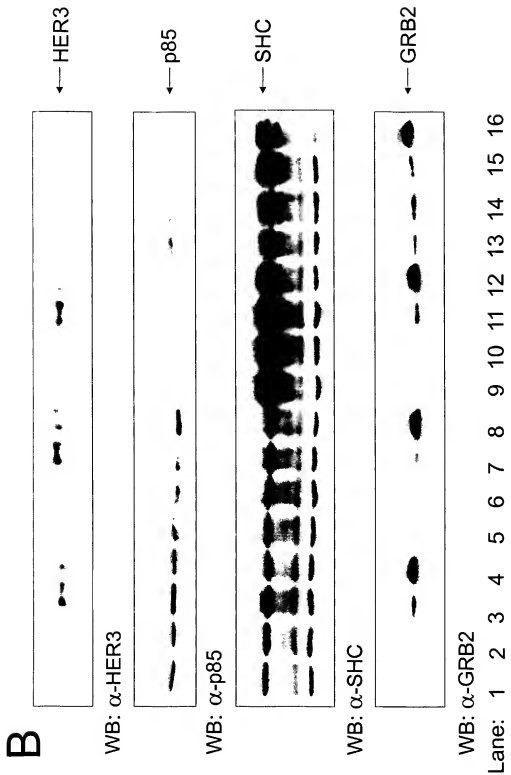


Figure1
Htun-van der Horst et al.

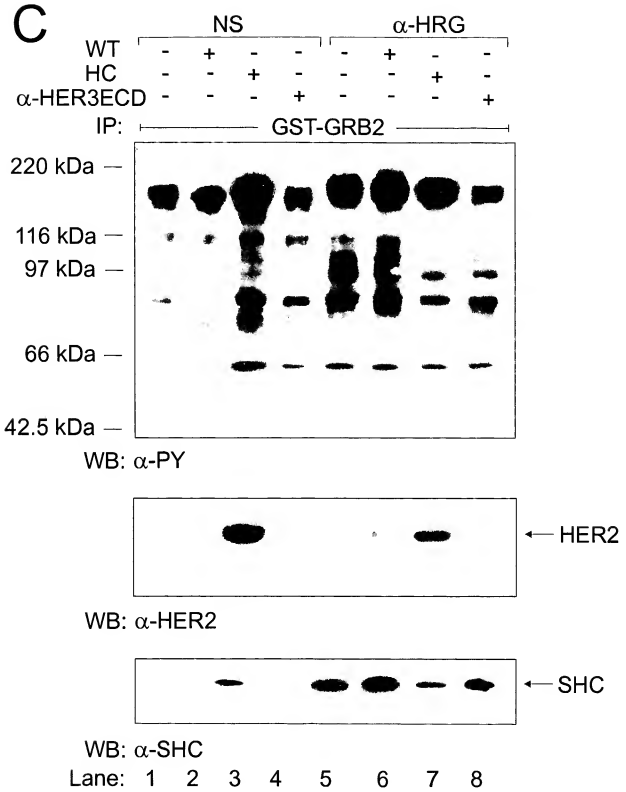


Figure 1
Htun-van der Horst et al.

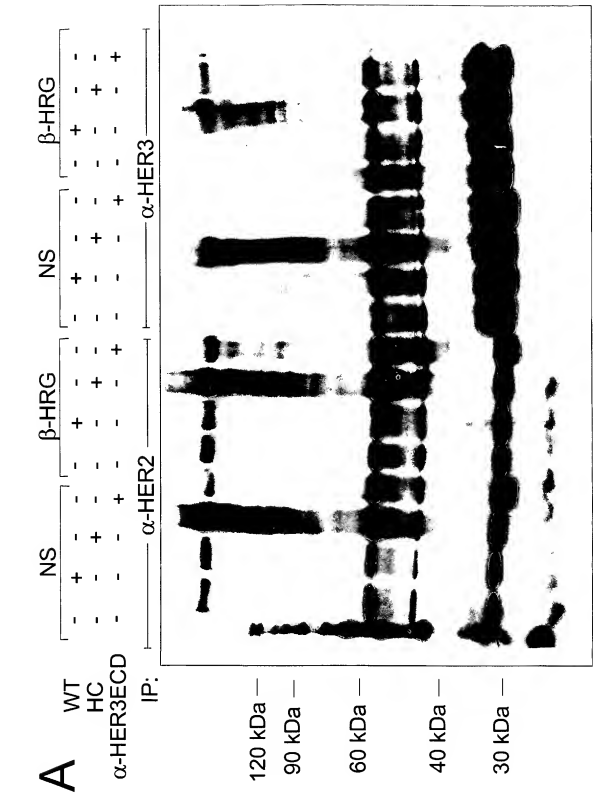


Figure2
Htun-van der Horst

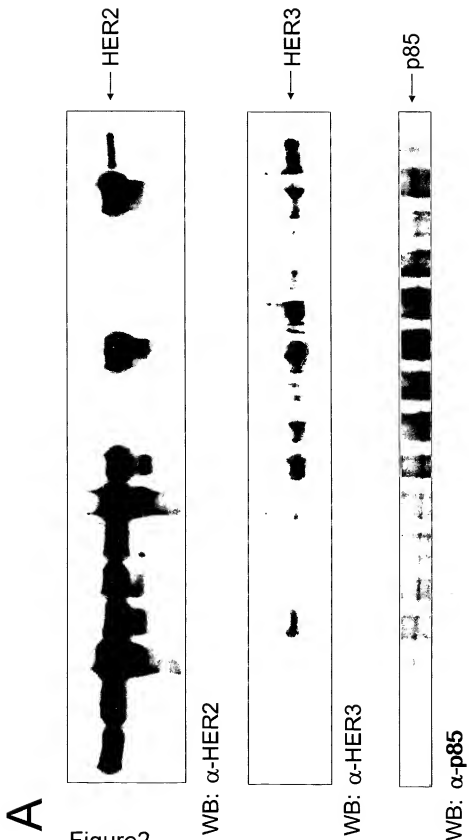


Figure2
Htun-van der Horst

Figure2
Htun-van der Horst

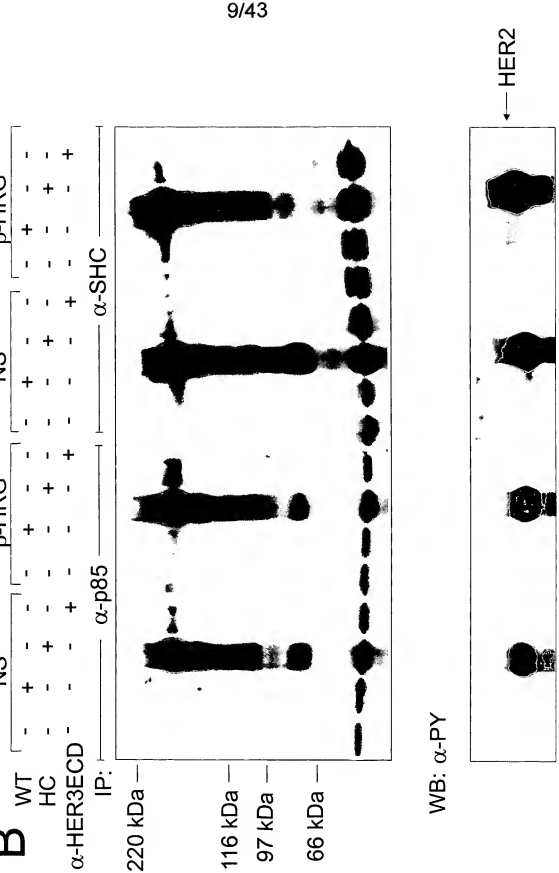


Figure 2
Htun-van der Horst et al.

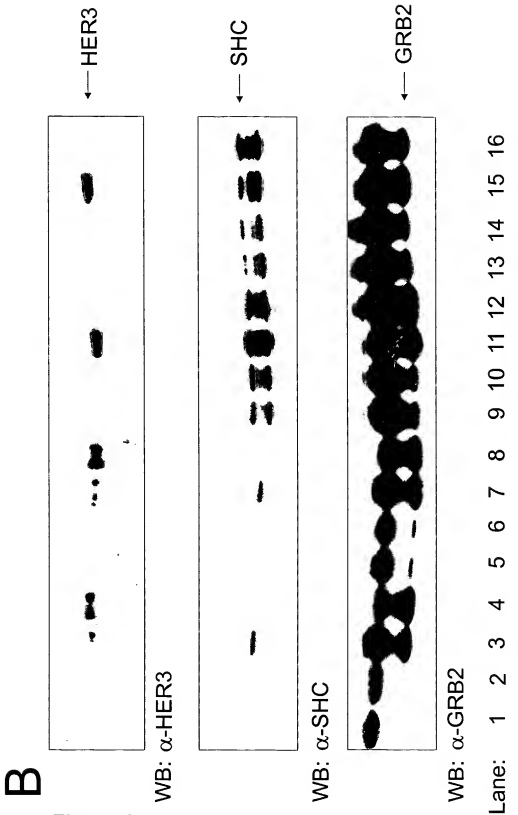


Figure 2
Htun-van der Horst et al.

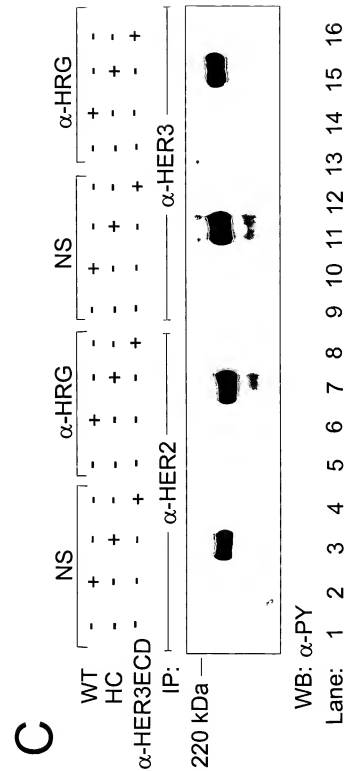


Figure 2
Htun-van der Horst et al.

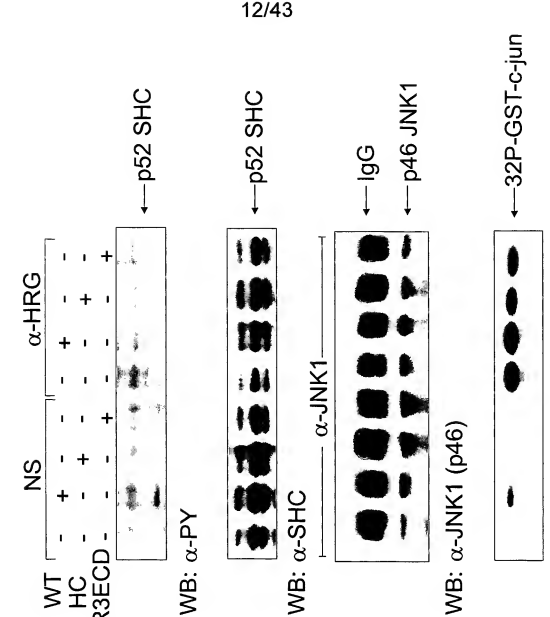


Figure 3
Htun-van der Horst et al.

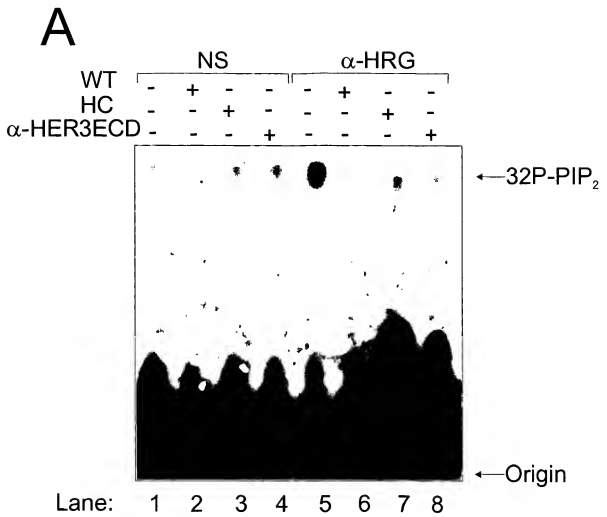


Figure 3
Htun-van der Horst et al.

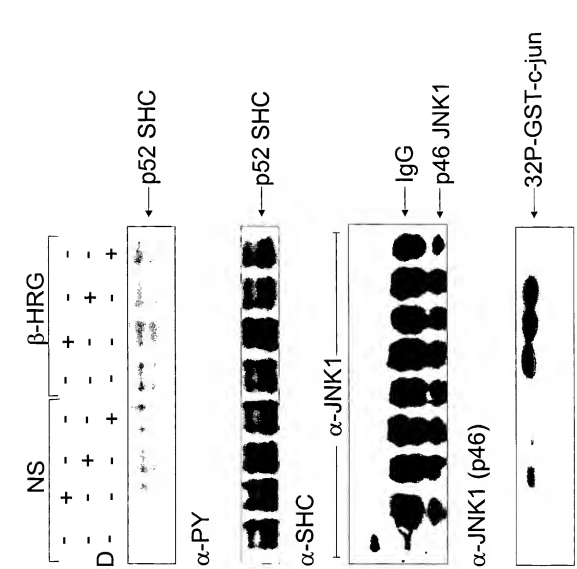


Figure 3
Htun-van der Horst et al.

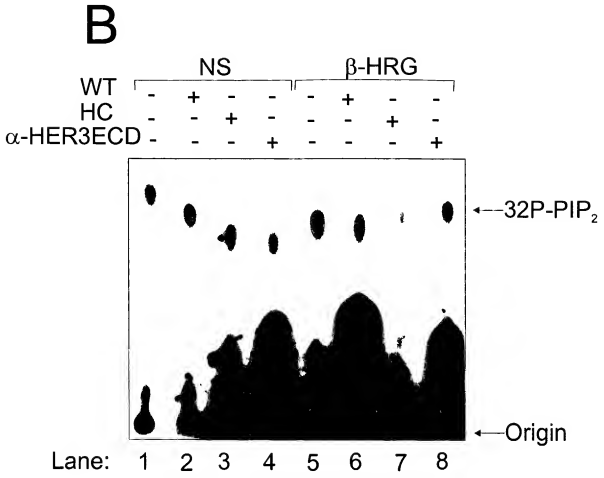


Figure 3
Htun-van der Horst et al.

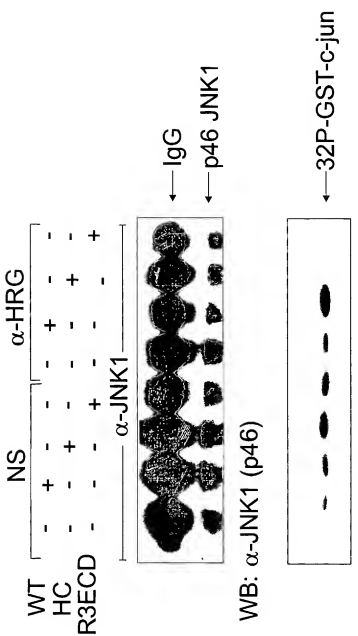


Figure 3
Htun-van der Horst et al.

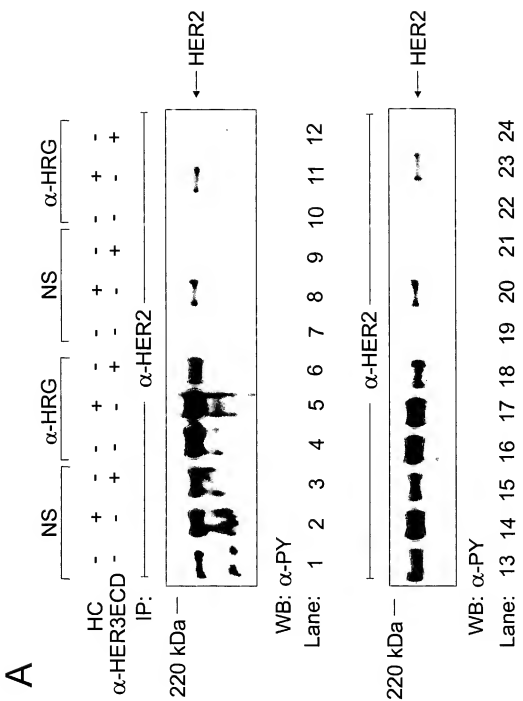
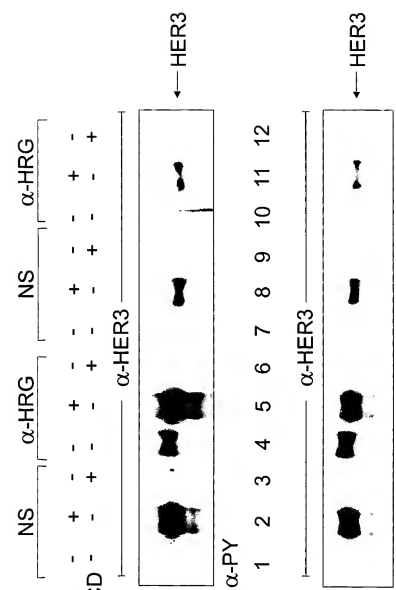


Figure 4
Htun-van der Horst et al.



Lane 1-6	Lane 7-12	Lane 13-18	Lane 19-24
HER2-WT	HER2-KM	HER2-WT	HER2-KM
HER3-WT	HER3-WT	HER3-KM	HER3-KM

Figure 4
Htun-van der Horst et al.

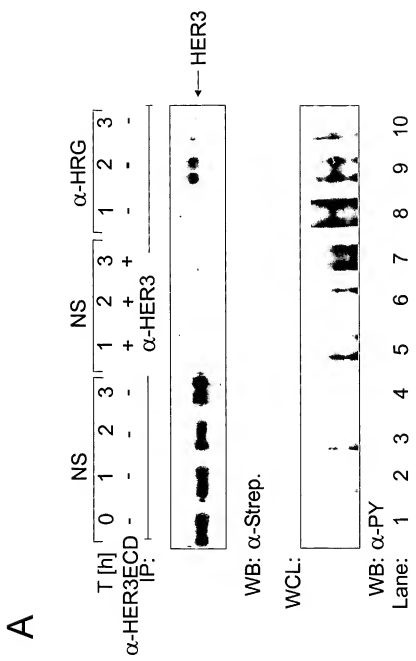


Figure 5
Htun-van der Horst et al.

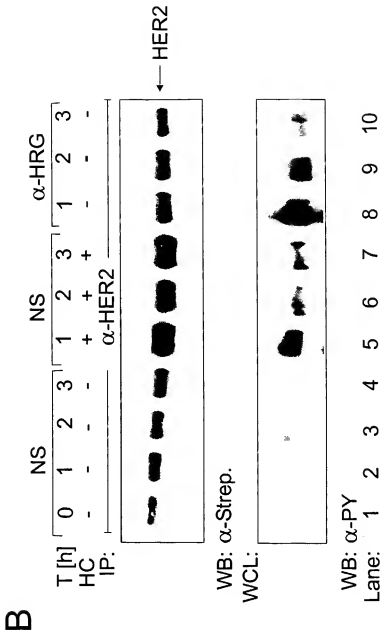


Figure 5
Htun-van der Horst et al.

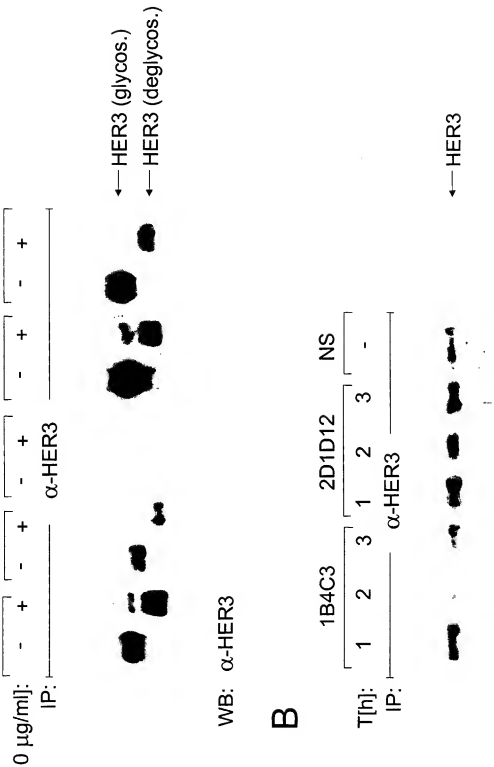


Figure 6
Htun van der Horst et al.

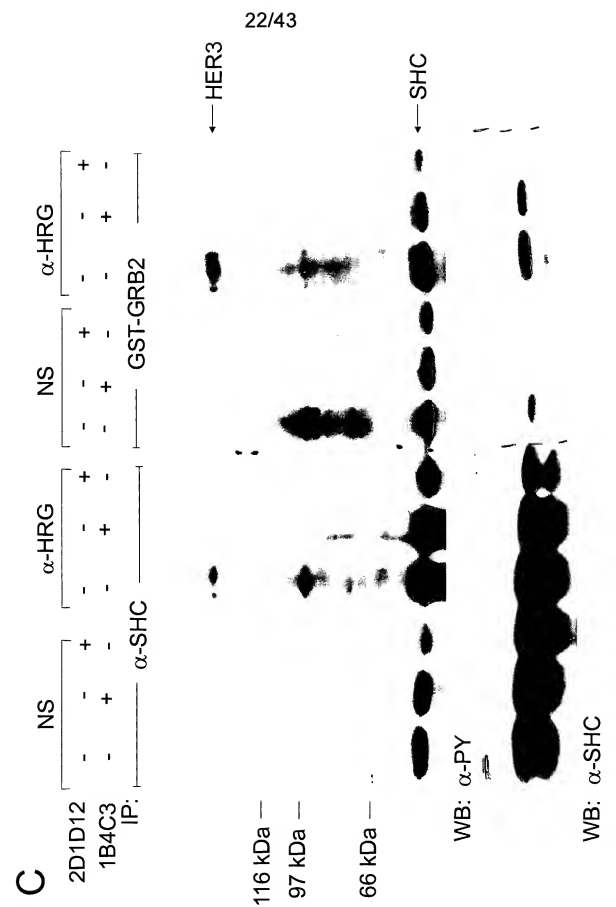


Figure 6
Htun van der Horst et al.

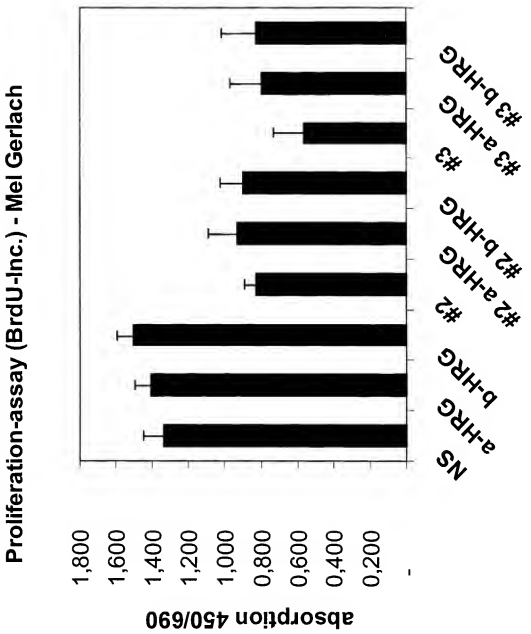


Figure 7
Htun van der Horst et al.

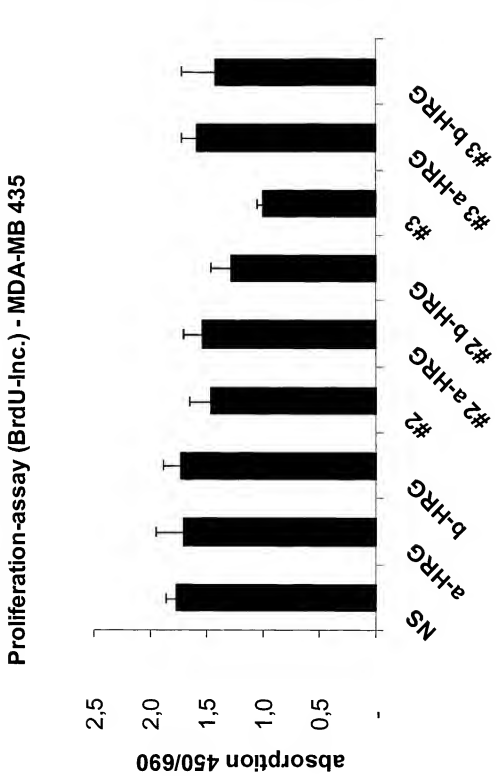


Figure 7
Htun van der Horst et al.

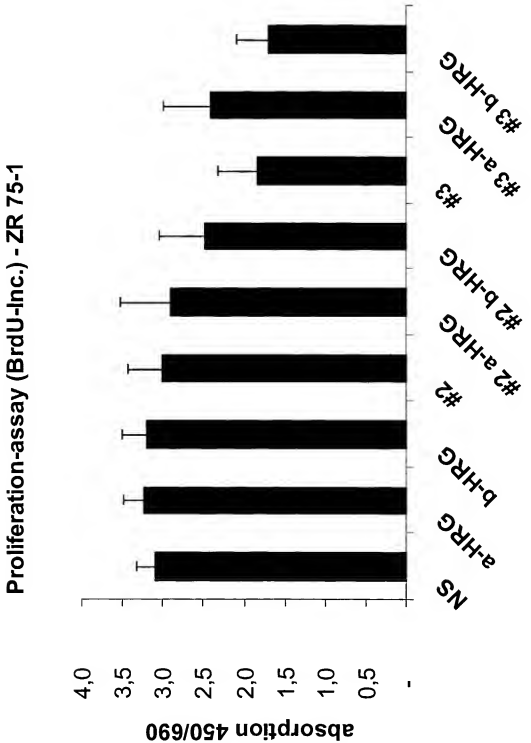


Figure 7
Htun van der Horst et al.

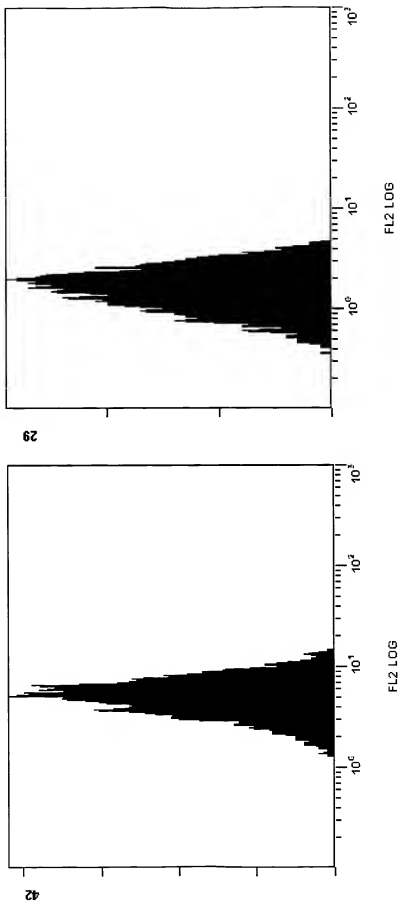
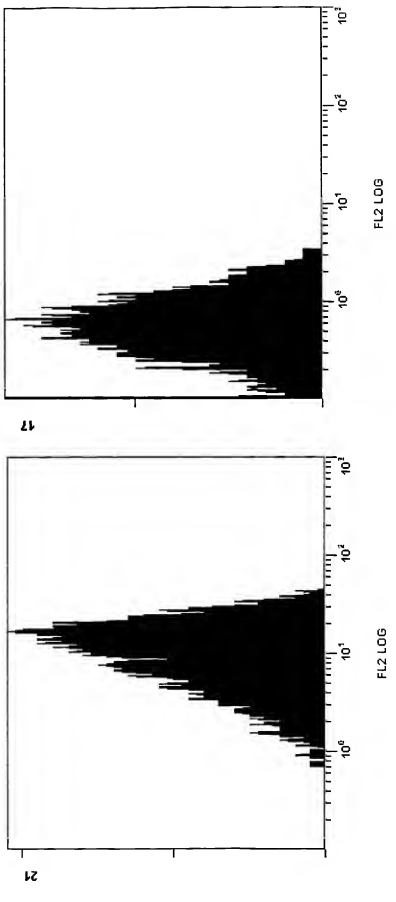


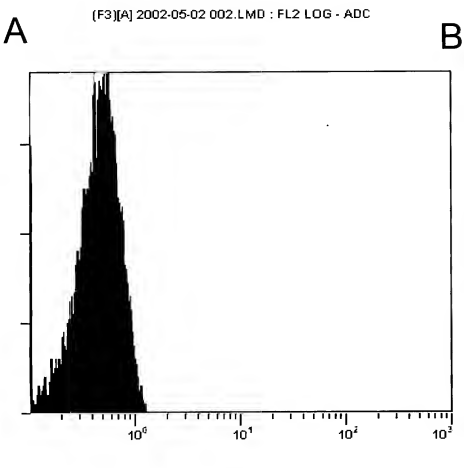
Figure8
Htun van der Horst et al.



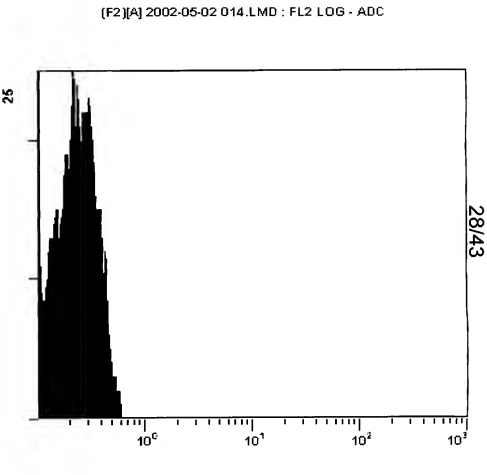
HER3
MDA-MB-435S

HER3
Mel Juso

Figure8
Htun van der Horst et al.



Myc (control)
MDA-MB-435S



Myc (control)
Mel Juso

Figure8
Htun van der Horst et al.

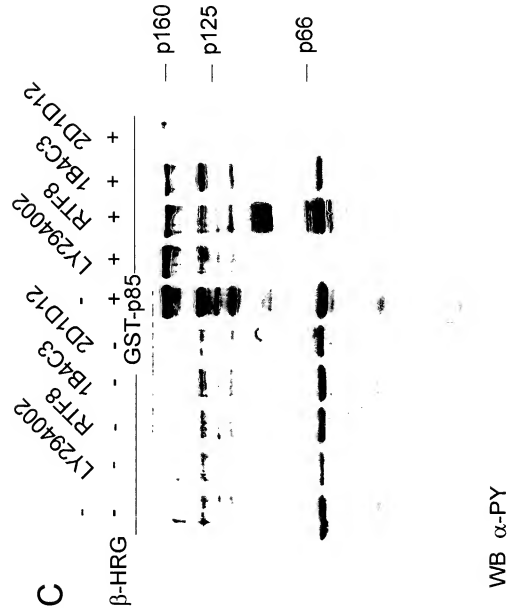


Figure 8
Supplementary Data
Htun van der Horst et al.

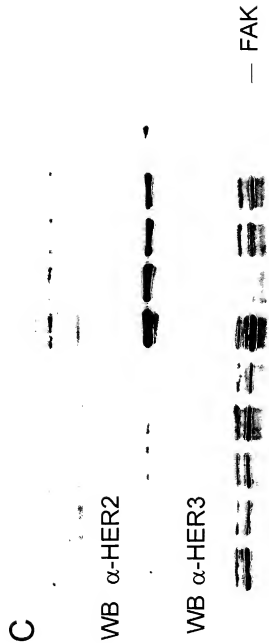


Figure 8
Supplementary Data
Htun van der Horst et al.

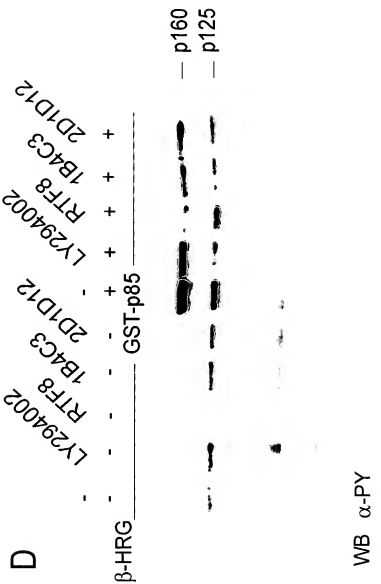


Figure 8
Supplementary Data
Htun van der Horst et al.

Figure 8
Supplementary Data
Htun van der Horst et al.

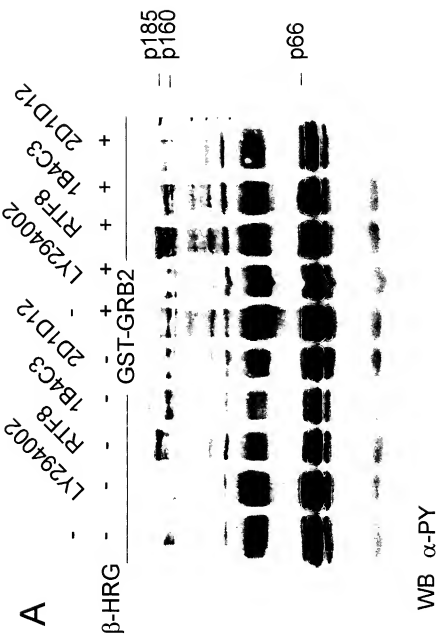


Figure 9
Supplementary Data
Htun van der Horst et al.

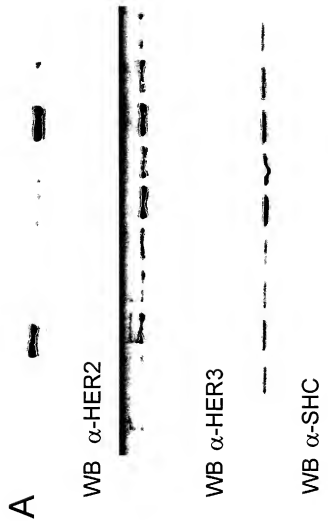


Figure 9
Supplementary Data
Htun van der Horst et al.

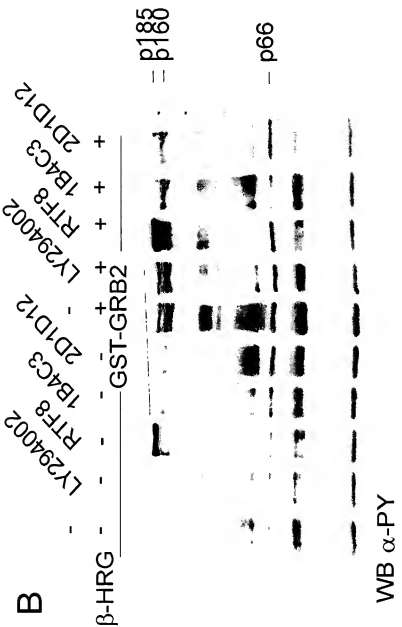


Figure 9
Supplementary Data
Htun van der Horst et al.

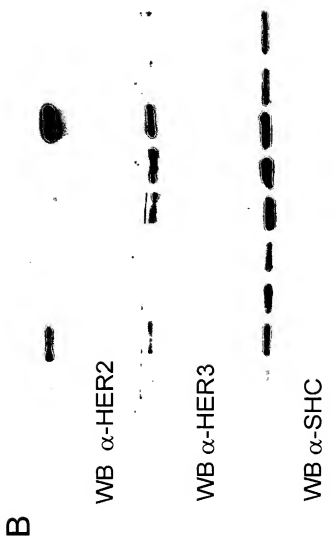


Figure 9
Supplementary Data
Htun van der Horst et al.

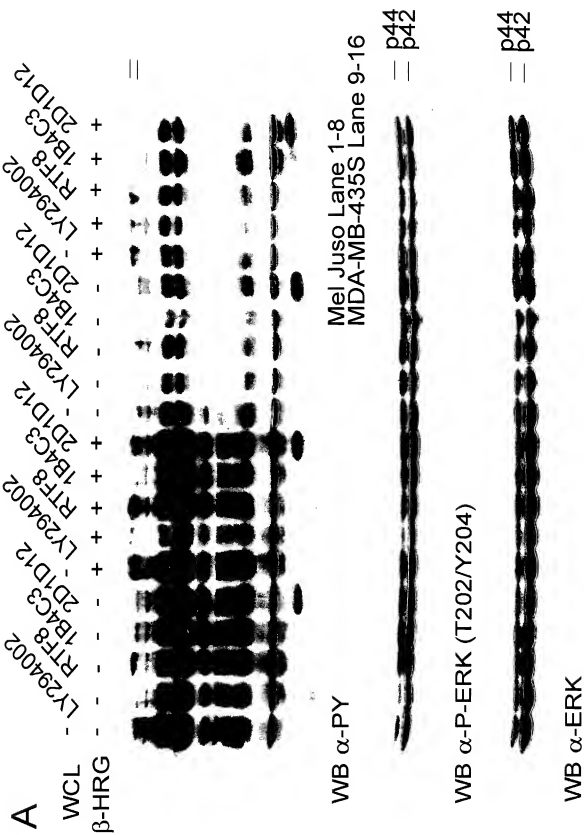


Figure 10
Supplementary Data
Htun van der Horst et al.

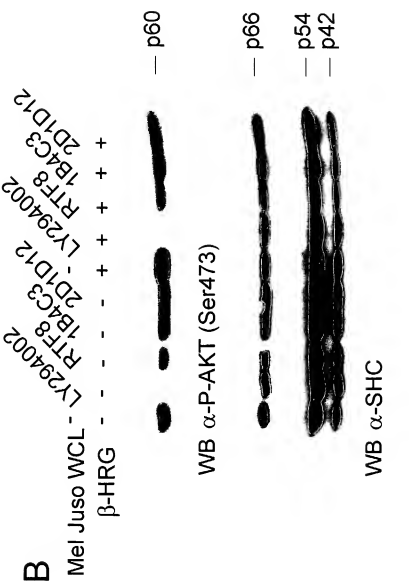


Figure 10
Supplementary Data
Htun van der Horst et al.

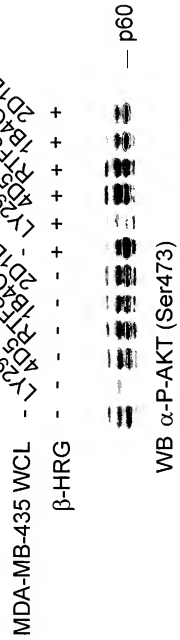


Figure 10
Supplementary Data
Htun van der Horst et al.

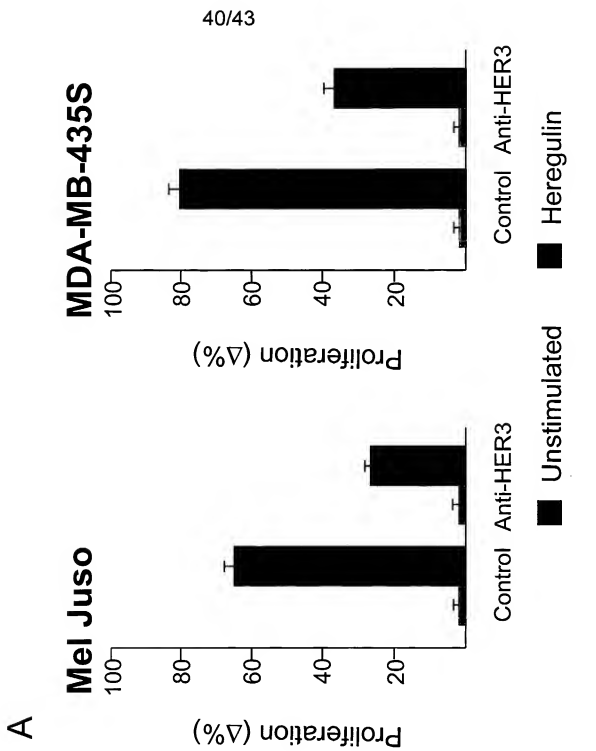


Figure 11
Supplementay Data
Htun van der Horst et al.

B

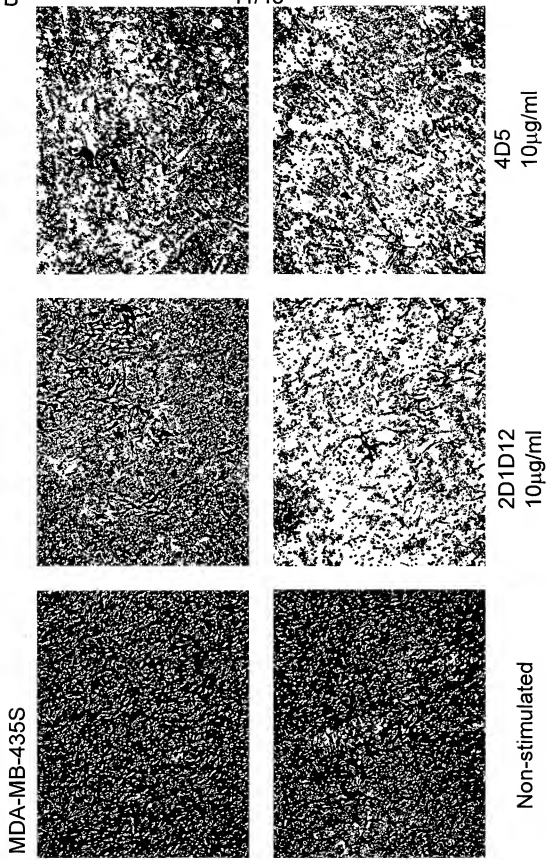


Figure11
Supplementary Data
Htun van der Horst et al.

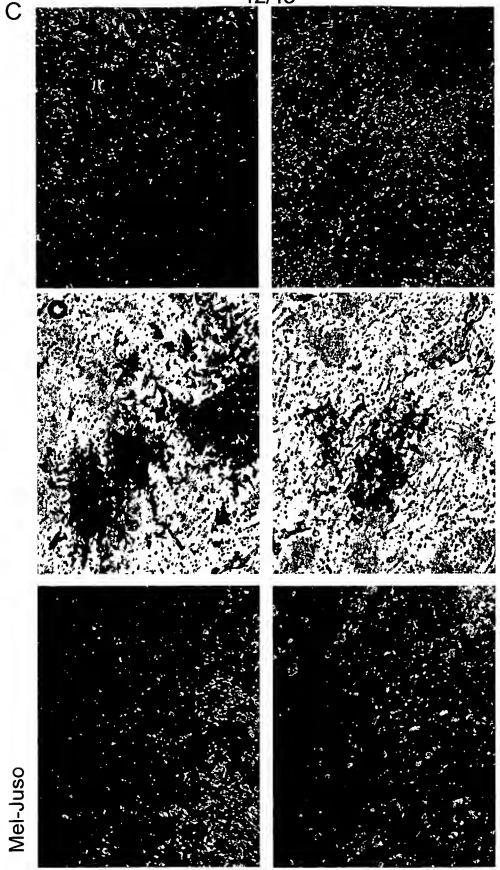


Figure11
Supplementary Data
Htun van der Horst et al.

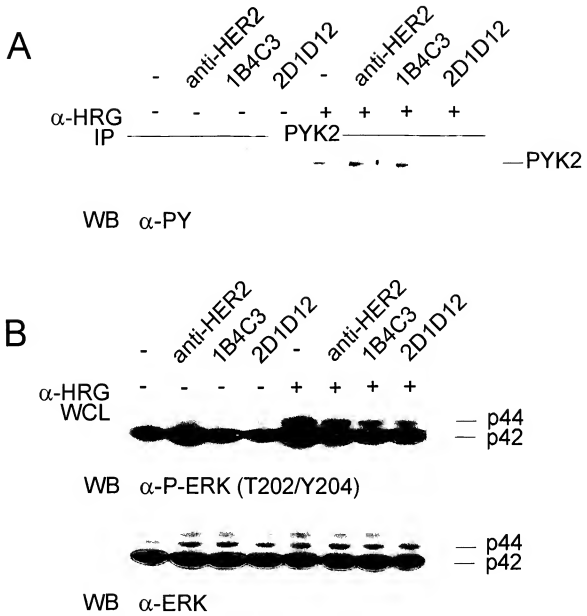


Figure 12
Supplementary Data
Htun van der Horst et al.

INTERNATIONALES FORMBLATT

Max-Planck-Institut
für Biochemie
Am Klopferspitz 18 a

82152 Martinsried

EMPFANGSBESTÄTIGUNG BEI ERSTHINTERLEGUNG, ausgestellt gemäß Regel 7.1 von der unten angegebenen INTERNATIONALEN HINTERLEGUNGSSTELLE

I. KENNZEICHNUNG DES MIKROORGANISMUS

Vom HINTERLEGER zugeteiltes Bezugszeichen:	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER:
2D1D12	DSM ACC2517

II. WISSENSCHAFTLICHE BESCHREIBUNG UND/ODER VORGESCHLAGENE TAXONOMISCHE BEZEICHNUNG

Mit dem unter I. bezeichneten Mikroorganismus wurde

(X) eine wissenschaftliche Beschreibung
( ) eine vorgeschlagene taxonomische Bezeichnung

eingereicht.
(Zutreffendes ankreuzen).

III. EINGANG UND ANNAHME

Diese internationale Hinterlegungsstelle nimmt den unter I bezeichneten Mikroorganismus an, der bei ihr am 2001-07-24 (Datum der Ersthinterlegung)[1] eingegangen ist.

IV. EINGANG DES ANTRAGS AUF UMWANDLUNG

Der unter I bezeichnete Mikroorganismus ist bei dieser Internationalen Hinterlegungsstelle am eingegangen (Datum der Ersthinterlegung) und ein Antrag auf Umwandlung dieser Ersthinterlegung in eine Hinterlegung gemäß Budapester Vertrag ist am eingegangen (Datum des Eingangs des Antrags auf Umwandlung).

V. INTERNATIONALE HINTERLEGUNGSSTELLE

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Unterschrift(en) der zur Vertretung der Internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten:
Anschrift: Mascheroder Weg 1b D-38124 Braunschweig	Datum: 2001-07-31

[1] Falls Regel 6.4 Buchstabe d zutrifft, ist dies der Zeitpunkt, zu dem der Status einer internationalen Hinterlegungsstelle erworben worden ist.

INTERNATIONALES FORMBLATT

Max-Planck-Institut
für Biochemie
Am Klopferspitz 18 a

82152 Martinsried

LEBENSFÄHIGKEITSBESCHEINIGUNG
ausgestellt gemäß Regel 10.2 von der unten angegebenen
INTERNATIONALEN HINTERLEGUNGSSTELLE

I. HINTERLEGER	II. KENNZEICHNUNG DES MIKROORGANISMUS
Name: Max-Planck-Institut für Biochemie Anschrift: Am Klopferspitz 18 a 82152 Martinsried	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER: DSM ACC2517 Datum der Hinterlegung oder Weiterleitung[1]: 2001-07-24

III. LEBENSFÄHIGKEITSBESCHEINIGUNG

Die Lebensfähigkeit des unter II genannten Mikroorganismus ist am 2001-07-24 [1] geprüft worden.
Zu diesem Zeitpunkt war der Mikroorganismus

(X)[2] lebensfähig
( )[2] nicht mehr lebensfähig

IV. BEDINGUNGEN, UNTER DENEN DIE LEBENSFÄHIGKEITSPRÜFUNG DURCHGEFÜHRT WORDEN IST[4]

V. INTERNATIONALE HINTERLEGUNGSSTELLE

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Anschrift: Mascheroder Weg 1b D-38124 Braunschweig	Unterschrift(en) der zur Vertretung der internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten: V. Weiß Datum: 2001-07-31

[1] Angabe des Datums der Ersthinterlegung. Wenn eine erneute Hinterlegung oder eine Weiterleitung vorgenommen worden ist, Angabe des Datums der jeweils letzten erneuten Hinterlegung oder Weiterleitung.
[2] In den in Regel 10.2 Buchstabe a Ziffer ii und iii vorgesehenen Fällen Angabe der letzten Lebensfähigkeitsprüfung.
[3] Zutreffendes ankreuzen.
[4] Ausfüllen, wenn die Angaben beantragt worden sind und wenn die Ergebnisse der Prüfung negativ waren.

INTERNATIONALES FORMBLATT

Max-Planck-Institut
für Biochemie
Am Klopferspitz 18A
82152 Martinsried

EMPFANGSBESTÄTIGUNG BEI ERSTHINTERLEGUNG,
ausgestellt gemäß Regel 7.1 von der unten angegebenen
INTERNATIONALEN HINTERLEGUNGSSTELLE

I. KENNZEICHNUNG DES MIKROORGANISMUS

Vom HINTERLEGER zugeteiltes Bezugszeichen:	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER:
1B4C3	DSM ACC2527

II. WISSENSCHAFTLICHE BESCHREIBUNG UND/ODER VORGESCHLAGENE TAXONOMISCHE BEZEICHNUNG

Mit dem unter I. bezeichneten Mikroorganismus wurde

(X) eine wissenschaftliche Beschreibung
( ) eine vorgeschlagene taxonomische Bezeichnung

eingereicht.
(Zutreffendes ankreuzen).

III. EINGANG UND ANNAHME

Diese internationale Hinterlegungsstelle nimmt den unter I bezeichneten Mikroorganismus an, der bei ihr am 2001-09-04 (Datum der Ersthinterlegung)[1] eingegangen ist.

IV. EINGANG DES ANTRAGS AUF UMWANDLUNG

Der unter I bezeichnete Mikroorganismus ist bei dieser Internationalen Hinterlegungsstelle am eingegangen (Datum der Ersthinterlegung) und ein Antrag auf Umwandlung dieser Ersthinterlegung in eine Hinterlegung gemäß Budapester Vertrag ist am eingegangen (Datum des Eingangs des Antrags auf Umwandlung).

V. INTERNATIONALE HINTERLEGUNGSSTELLE

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Unterschrift(en) der zur Vertretung der internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten:
Anschrift: Mascheroder Weg 1b D-38124 Braunschweig	U. Wirth
	Datum: 2001-09-11

[1] Falls Regel 6.4 Buchstabe d zutrifft, ist dies der Zeitpunkt, zu dem der Status einer internationalen Hinterlegungsstelle erworben worden ist.

INTERNATIONALES FORMBLATT

Max-Planck-Institut
für Biochemie
Am Klopferspitz 18A
82152 Martinsried

LEBENSFÄHIGKEITSBESCHEINIGUNG
ausgestellt gemäß Regel 10.2 von der unten angegebenen
INTERNATIONALEN HINTERLEGUNGSSTELLE

I. HINTERLEGER	II. KENNZEICHNUNG DES MIKROORGANISMUS
Name: Max-Planck-Institut für Biochemie Anschrift: Am Klopferspitz 18A 82152 Martinsried	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER: DSM ACC2527 Datum der Hinterlegung oder Weiterleitung[1]: 2001-09-04

III. LEBENSFÄHIGKEITSBESCHEINIGUNG

Die Lebensfähigkeit des unter II genannten Mikroorganismus ist am 2001-09-04 [2] geprüft worden.
Zu diesem Zeitpunkt war der Mikroorganismus

(X)[3] lebensfähig
( )[3] nicht mehr lebensfähig

IV. BEDINGUNGEN, UNTER DENEN DIE LEBENSFÄHIGKEITSPRÜFUNG DURCHGEFÜHRT WORDEN IST[4]

V. INTERNATIONALE HINTERLEGUNGSSTELLE	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Anschrift: Mascheroder Weg 1b D-38124 Braunschweig	Unterschrift(en) der zur Vertretung der internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten: V. Weiss Datum: 2001-09-11

[1] Angabe des Datums der Ersthinterlegung. Wenn eine erneute Hinterlegung oder eine Weiterleitung vorgenommen worden ist, Angabe des Datums der jeweils letzten erneuten Hinterlegung oder Weiterleitung.
[2] In den in Regel 10.2 Buchstabe a Ziffer ii und iii vorgesehenen Fällen Angabe der letzten Lebensfähigkeitsprüfung.
[3] Zutreffendes ankreuzen.
[4] Ausfüllen, wenn die Angaben beantragt worden sind und wenn die Ergebnisse der Prüfung negativ waren.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/08938

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 A61K47/48 G01N33/577 G01N33/574 A61P35/00
G01N33/50 C12N5/20 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R. VADLAMUDI ET AL.: "Regulation of cyclooxygenase-2 pathway by HER2 receptor." ONCOGENE, vol. 18, no. 2, 14 January 1999 (1999-01-14), pages 305-314, XP001030629 Basingstoke, GB abstract page 307, left-hand column, line 19 - right-hand column, line 12 page 307, right-hand column, line 36 - page 309, left-hand column, line 11 page 312, left-hand column, line 2 - line 38 page 312, right-hand column, line 3 - line 26 --- -/--	1,2,5-9, 11-15, 17-22, 24-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 2002	06/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 31048 A (WARNER-LAMBERT COMPANY) 2 June 2000 (2000-06-02) claims ---	1,4,13, 14,17, 18,20, 21,24-26
X	WO 97 35885 A (GENENTECH, INC.) 2 October 1997 (1997-10-02) cited in the application page 28, line 27 -page 29, line 24 example claims ---	1,4-8, 12-15, 17-22, 24-26
X	WO 00 78347 A (THE VICTOR CHANG CARDIAC RESEARCH INSTITUTE) 28 December 2000 (2000-12-28) claims page 16, line 25 - line 29 ---	1,5-8, 11-15, 17-22, 24-26
X	US 5 804 396 A (PLOWMAN) 8 September 1998 (1998-09-08) claims ---	25,26
A	D. BAECKSTRÖM ET AL.: "Morphogenetic and proliferative responses to heregulin of mammary epithelial cells in vitro are dependent on HER2 and HER3 and differ from the responses to HER2 homodimerisation or hepatocyte growth factor." INTERNATIONAL JOURNAL OF ONCOLOGY, vol. 16, no. 6, June 2000 (2000-06), pages 1081-1090, XP001030626 Greece page 1084, right-hand column, line 4 - line 28 ---	1-27,29
A	D. TODD ET AL.: "Ionizing radiation stimulates existing signal transduction pathways involving the activation of epidermal growth factor receptor ans erbB-3, and changes of intracellular calcium in A431 human squamous carcinoma cells." JOURNAL OF RECEPTOR AND SIGNAL TRANSDUCTION RESEARCH, vol. 19, no. 6, November 1999 (1999-11), pages 885-908, XP001030590 USA page 897, line 15 - line 18 ---	1-27,29
A	US 5 480 968 A (KRAUS ET AL.) 2 January 1996 (1996-01-02) column 9, line 59 -column 11, line 52 --- -/--	1-27,29

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	T. RAJKUMAR ET AL.: "A monoclonal antibody to the human c-erbB3 protein stimulates the anchorage-independent growth of breast cancer cell lines." BRITISH JOURNAL OF CANCER, vol. 70, 1994, pages 459-465, XP000676619 GB cited in the application abstract discussion ---	1-27,29
T	US 6 277 640 B1 (BENNETT ET AL.) 21 August 2001 (2001-08-21) examples claims -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/08938

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 21-24 (partially) are directed to a method of treatment of the human/animal body, and although claims 21-24 (partially) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0031048 A	02-06-2000	AU 6261299 A	13-06-2000
		BG 105608 A	31-01-2002
		BR 9915487 A	31-07-2001
		CN 1330642 T	09-01-2002
		CZ 20011759 A3	16-01-2002
		EP 1131304 A1	12-09-2001
		HU 0104211 A2	29-05-2002
		JP 2002530386 T	17-09-2002
		NO 20012465 A	13-07-2001
		PL 347717 A1	22-04-2002
		SK 6572001 A3	05-02-2002
		WO 0031048 A1	02-06-2000
		US 6344455 B1	05-02-2002
WO 9735885 A	02-10-1997	AU 727082 B2	30-11-2000
		AU 2071197 A	17-10-1997
		BR 9708343 A	03-08-1999
		CA 2246429 A1	02-10-1997
		CN 1214695 A	21-04-1999
		EP 0896586 A1	17-02-1999
		JP 2000508526 T	11-07-2000
		NZ 331360 A	27-03-2000
		WO 9735885 A1	02-10-1997
		ZA 9702554 A	25-09-1998
WO 0078347 A	28-12-2000	WO 0078347 A1	28-12-2000
		AU 5199900 A	09-01-2001
		EP 1187634 A1	20-03-2002
US 5804396 A	08-09-1998	NONE	
US 5480968 A	02-01-1996	US 5183884 A	02-02-1993
		US 5820859 A	13-10-1998
		US 5916755 A	29-06-1999
		AT 163970 T	15-03-1998
		AU 654805 B2	24-11-1994
		AU 6889991 A	26-06-1991
		DE 69032133 D1	16-04-1998
		DE 69032133 T2	02-07-1998
		DK 502927 T3	02-06-1998
		EP 0502927 A1	16-09-1992
		ES 2113877 T3	16-05-1998
		JP 6503467 T	21-04-1994
		JP 3196079 B2	06-08-2001
		JP 2001299373 A	30-10-2001
		WO 9108214 A1	13-06-1991
US 6277640 B1	21-08-2001	AU 7700301 A	13-02-2002
		WO 0210409 A1	07-02-2002